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# BRIEF COMMUNICATIONS

## STEM CELL REGIONS IN FILIFORM PAPILLAE OF TONGUE AS TARGETS OF GRAFT-VERSUS-HOST DISEASE[1]

Epithelial stem cells are thought to reside in subpopulations of epithelium located in specific geographic sites within the epithelial structures. For example, in the skin, both the rete ridge of the epidermis and the parafollicular hair bulge of the pilar apparatus have been identified as sites where label-retaining cells may be found in tritiated thymidine experiments. The retention of label is taken to suggest that slowly cycling cells are present, identifying these as sites of epithelial stem cells (*1, 2*). In 1985, we reported evidence that graft-versus-host disease (GVHD)* attacks the rete ridge area of the epidermis. Recently, we have also shown this in the parafollicular hair bulge in humans after Murphy et al. reported this region to be highly selected for attack in GVHD in mice (*3, 4*). Similarly, stem cells may be present in the squamocolumnar junction region of the mouse forestomach, which also appears to be a site of involvement of GVHD (*5, 6*). One of the more intriguing geographic localizations of epithelial stem cells is the anterior and posterior column base or shoulder of the filiform papilla of the tongue in mammals, particularly the hamster. We predicted that this region would be preferentially involved in canine and human GVHD, but we had great difficulty in demonstrating this in humans because of the difficulty of obtaining biopsy or autopsy tissue at the necessarily early time point in the disease process, as discussed below. In our studies of canine GVHD, we have recently encountered examples of early GVHD showing distinct evidence that the shoulders of the filiform papillae of the tongue are heavily involved in the process of GVHD.

Table 1 details information on 11 dogs, 7 of them allograft recipients, in which this observation was sought in the course of several studies of dogs undergoing marrow transplantation. Each of the 7 animals was given an allograft of bone marrow from a matched littermate or an unrelated mismatched donor after a single dose of 920 cGy total body irradiation, and treated with prophylactic drugs (e.g., succinyl acetone or FK506) variably effective in preventing GVHD (*7, 8*). Figures 1 and 2 are medium power photographs of filiform papillae of specimens of canine dorsal tongue showing extensive involvement of the basal shoulder of the filiform papillae with a lymphocytic infiltrate and apoptotic bodies. These basal anterior and posterior shoulder loci are the regions of the filiform papilla that have been shown to be sites of stem cells based on label-retaining experiments with tritiated thymidine (*9*). Tongue specimens from 4 recipients of autologous marrow were available for comparison to the allografts. All dogs received the same 920 cGy radiation regimen. We found no tongue lesions in any of these control cases. The cases were not randomized. They were selected cases in the sense that we sampled tongue at autopsy from several allografted dogs with GVHD (a relatively small minority of which showed the focal lesions we sought) and several autografted dogs dying of other causes with approximately the same post-transplant survival ranges.

Our search for these lesions took some time because of the difficulty of obtaining tissue early enough in the course of GVHD to determine whether there was any focality to the attack of lymphocytes on the shoulder regions of the papilla relevant to this question. In many other canine and human cases, involvement of the tongue was either so diffuse or so severe (because biopsied late) as to render the question unanswerable, or the tongue was not involved in an inflammatory process at all. The reason for the former problem is that if the disease is sampled late, both the filiform and the circumvallate papillae are obscured due to a licheniform process

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* Abbreviation: GVHD, graft-versus-host disease.

TABLE 1. Dogs in study with GVHD (allografts) and control autologous recipients[a]

| Dog | Day post transplant | Donor type | GVHD distribution |
|---|---|---|---|
| D114 | 14 | Unrelated mismatched allograft | Skin, liver, gut |
| D130 | 15 | Unrelated mismatched allograft | Skin, liver, gut |
| D036 | 10 | Matched allograft | Skin, liver, gut |
| D295 | 10 | Unrelated mismatched allograft | Liver, gut |
| D309 | 14 | Unrelated mismatched allograft | Skin, liver, gut |
| D595 | 14 | DLA nonidentical, sex mismatched allograft | Skin, stomach, gut |
| D171 | 42 | Matched allograft | Liver, gut |
| C943 | 19 | Autograft | None |
| C946 | 98 | Autograft | None |
| C954 | 26 | Autograft | None |
| D426 | 18 | Autograft | None |

[a] The experimental protocols and facilities used were approved by the Fred Hutchinson Cancer Research Center Animal Care and Use Committee, per guidelines stipulated in the Experimental Animal Welfare Act of 1985, administered through the National Institutes of Health.

FIGURE 1. Filiform papilla from tongue of dog D114 showing anterior and posterior shoulder involvement with moderately heavy infiltrate and destruction of some epithelial cells, particularly in the posterior shoulder at left (hematoxylin and eosin, ×250).

FIGURE 2. This figure shows another posterior shoulder region of dog D114 where extensive involvement almost obliterates the posterior shoulder of a filiform papilla (hematoxylin and eosin, ×250).

similar to that of kraurosis vulvi or lichen planus, in which rete ridges and other geographic characteristics of the epithelium are replaced (and therefore obscured) by acanthosis, atrophy, or even outright ulceration. The reason for the latter problem is that the tongue is not always involved in the process, which is not surprising, since even the more classically accepted sites of GVHD are not always involved. For example, any combination of skin, liver, or gut may be involved in an individual human or canine recipient with GVHD (as is evident in part in Table 1). For these reasons, even the present selected data required a more extensive prospective search in the dog, where we and others have considerable experience with GVHD (*10*).

In summary, our observations indicate that early GVHD of the tongue in both matched and unrelated allograft situations involves the stem cell region of the filiform papilla, as defined by scientists working in epithelial stem cell biology. This further buttresses our hypothesis that GVHD has a preference for stem cells or their early progeny.

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# TRANSPLANTATION IN HUMANS OF ENCAPSULATED XENOGENEIC CELLS WITHOUT IMMUNOSUPPRESSION

## A Preliminary Report[1]

Several investigators have reported experimental clinical allotransplantation of cells or dissociated tissue, including islets of Langerhans into the peritoneal cavity for the treatment of type 1 diabetes (*1*), fetal mesencephalic cells into the striatum for Parkinson's disease (*2*), and dissociated adrenal tissue into the intrathecal space for the relief of chronic pain (*3*). Despite positive clinical outcomes, widespread application of these approaches is limited by the scarcity of suitable human graft material in the same fashion that whole organ transplantation is currently constrained. In addition, recipients of these allogeneic cell transplants require immunosuppression. Over the past decade, techniques have been perfected to enclose small clusters of cells within implantable capsules formed by semipermeable membranes. Such membranes have pores that can be suitably sized to permit passage of nutrients and bioactive cell secretions, while restricting transit of immunoglobulins, lytic factors of the complement system, and immune competent cells. Several groups have advanced this technology to the point of successful application in animal models of diabetes (*4*), Parkinson's disease (*5*, *6*), and pain (*7*, *8*). Significantly, these membrane barriers have been shown to provide sufficient immune protection to prevent the rejection of encapsulated xenogeneic cells without immunosuppression in both rodents (*4*, *5*, *7*, *8*) and nonhuman primates (*6*). These results created the possibility of xenogeneic sourcing of cells for transplantation in encapsulated form to humans; we report here the first clinical demonstration that such xenotransplantation is feasible.

Encapsulated adrenal chromaffin cells implanted by laminectomy were found to significantly reduce the effects of acute (*7*) and chronic (*8*) pain in rodent models. The rationale for selecting chromaffin cells was their known release of a cocktail of analgesic substances, including catecholamines, enkephalins, endorphins, neurotensin, and somatostatin (*3*). We used sheep as a model to develop and validate an implant suitable for intrathecal implantation into the human by lumbar puncture and subsequent retrieval by a minor surgical procedure (*9*). A phase I population with intractable chronic pain secondary to terminal cancer was selected for initial clinical evaluation. The balance to patients of benefit to risk seemed favorable given the terminal nature of their disease, the severity of their pain, and the limited intervention required by intrathecal implantation with local anesthesia. The goal of the initial protocol was to confirm implant safety and xenogeneic cell viability in humans; pain relief was assessed but not intended as a formal outcome parameter. Nevertheless, because the procedure was invasive, we transplanted an amount of cells expected to provide some analgesic effect based upon earlier animal studies.

Cells for the implants were obtained from adrenal chromaffin cells harvested from 2-week-old calves obtained from healthy livestock herds routinely tested for adventitious agents and selected for absence of known bovine spongiform encephalitis. Cells were recovered by enzymatic digestion (*9*). After characterization, purified cells were suspended in an alginate matrix and introduced into the lumen of a modacrylic hollow fiber membrane with a molecular mass cutoff of approximately 50,000 daltons. The membrane was sealed at both ends and attached to a silicone tether, which facilitated handling and retrieval. Each implant, whose detailed fabrication is described elsewhere (*9*), was 5 cm long, 0.9 mm in diameter, and contained approximately 2 million cells. Devices were released for implantation only after individual qualification for sterility and release of catecholamines. Nicotine (63 μM) evoked norepinephrine release of the capsules before implantation ranged from 2.5 to 5.0 nM/2 ml/30 min. The protocol called for a 30-day study that could be extended to a maximum of 90 days upon the request of the patient. Three patients met the following criteria: terminal cancer, pain incompletely relieved by narcotic therapy, and no evidence of active infection or tumor in the meningeal space. After informed consent was granted by the patients and approval was received from the Ethical Committee of the Faculty of Medicine of the University of Lausanne, Switzerland, the devices were implanted under local anesthesia at the $L_3$-$L_4$ level through a catheter using a cannula introducer system (*9*). The membrane portion of device rested in the lumbar subarachnoid space, whereas the free end of the silicone tether was anchored to the subcutaneous tissue and completely covered with a skin closure.

Postoperative recovery was uneventful, except for 1 patient who experienced postpunctural headaches of several days duration. Patients 2 and 3 sharply reduced their intake of narcotics and analgesics. Patient 1 decreased his morphine intake but had to be treated with others analgesic medication, including epidural fentanyl and local anesthetics, to

[1] The clinical protocols were reviewed and approved by the Ethical Committee of the Faculty of Medicine of the University of Lausanne, Switzerland. All patients granted informed consent. Portions of the preclinical or clinical protocol involving animals were reviewed and approved by the Committee on Animal Experimentation of the canton of Vaud, Lausanne, Switzerland.